

**1869-Pos****Effects of Dietary Lipids on Stress-Dependent Depolarization of Cardiac Mitochondrial Membrane**Aristide C. Chikando<sup>1,2</sup>, Ramzi Khairallah<sup>2</sup>, William Stanley<sup>2</sup>, W. Jonathan Lederer<sup>1,2</sup>.<sup>1</sup>University of Maryland Biotechnology Institute, Baltimore, MD, USA,<sup>2</sup>University of Maryland School of Medicine, Baltimore, MD, USA.

Mitochondria play an important role in cardiac cellular physiology by generating ATP, contributing to the production and scavenging of reactive O<sub>2</sub> species (ROS) and by influencing intracellular Ca<sup>2+</sup>. It has been hypothesized that dietary fatty acids (FA), specifically polyunsaturated FA, can affect mitochondrial function including those noted above. We have examined how FAs may influence mitochondrial membrane potential ( $\Delta\Psi_{\text{mito}}$ ) measured with tetramethyl rhodamine methyl ester (TMRM) when the mitochondria are stressed by light at 543 nm. We examined the time-course of photon-stress on  $\Delta\Psi_{\text{mito}}$  in single ventricular myocytes from rats on a lipid-restricted diet. The stress-illumination was limited to a square region of a single cell that covered about 20% of the cell cross-section. The illumination was repeated at 3 second intervals over the period of an hour. The repeated illumination led to gradual depolarization of mitochondria within the imaged region, the rate of which was distinct in each dietary group (see Fig. 1). The effects of photon-stress are altered by cyclosporin A and N-acetyl cysteine and these will be discussed. Measurement of the rate of oxygen consumption showed that depolarized mitochondria respired well but were uncoupled.

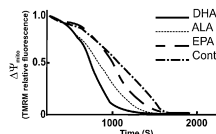
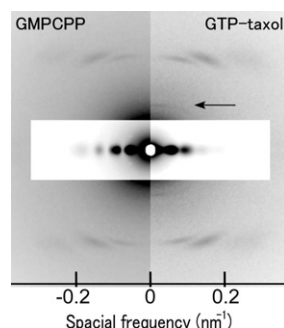


Fig. 1- Time-course of mitochondrial depolarization in the imaged region. Each curve represents the mean of 4 time-courses recorded in cells taken from the hearts of an animal (4 control and study groups) fed a control diet (barley) or a diet enriched with DHA (solid), EPA (dotted) and ALA (dash-dot). Each curve is significantly different from the other curves (P<0.05).

**Microtubules & Microtubule-associated Proteins****1870-Pos****Structural Changes of Microtubules During GTP Hydrolysis Revealed by X-Ray Fiber-Diffraction**Shinji Kamimura<sup>1</sup>, Hiroyuki Iwamoto<sup>2</sup>, Daisuke Miyashiro<sup>3</sup>.<sup>1</sup>Dept. Biol. Sci., Chuo Univ., Tokyo, Japan, <sup>2</sup>Research & Utilization Division, SPring-8, Sayo, Hyogo, Japan, <sup>3</sup>Dept. Life Sci., Univ. Tokyo, Komaba, Tokyo, Japan.

Microtubule (MT) is one of the most essential cytoskeletal elements in the eukaryote cells, which supports the mitosis, cell architecture and motility as well as the intracellular transportation. Tubulin dimers in a GTP-binding state (GTP-tubulin) are assembled onto MT-ends and make them stable. However, the following slow hydrolysis of GTP to GDP occurring in  $\beta$ -tubulin is assumed to weaken the lateral interactions among protofilaments and MTs in an uncapped state (MT-ends without GTP-tubulin) disassemble and shrink quickly. Such features of dynamic instability would be closely related to various MT functions. In the present study, we tested how the GTP-hydrolysis was related to the structural changes of MTs by X-ray fiber diffraction analysis (BL45XU, SPring-8,  $\lambda=0.09$  nm). By comparing diffraction peaks between GMPCPP- and GTP-taxol-MT, we found that 8 nm meridional reflection intensity (arrow in the figure) appeared only in GTP-taxol-MTs, which are assumed to consist mostly of GDP-tubulin. It would reflect some structural differences between GDP- and GTP-tubulin in situ. The change of tubulin-pitch by GTP-hydrolysis was also found. This is the first demonstration to show structural changes of MTs under physiological conditions.

**1871-Pos****Protein Friction Limits Diffusive and Directed Movements of Kinesin Motors on Microtubules**Volker Bormuth<sup>1</sup>, Vladimir Varga<sup>1</sup>, Jonathon Howard<sup>1</sup>, Erik Schäffer<sup>2</sup>.<sup>1</sup>Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, <sup>2</sup>TU-Dresden, Biotec, Dresden, Germany.

Friction limits the operation of macroscopic engines and is critical to the performance of micromechanical devices. We report measurements of friction in a biological nanomachine. Using optical tweezers, we characterized the frictional drag force of individual kinesin-8 motor proteins interacting with their microtubule tracks. At low speeds and with no energy source, the frictional drag was related to the diffusion coefficient by the Einstein relation. At higher

speeds, the frictional drag force increased nonlinearly, consistent with the motor jumping 8 nanometers between adjacent tubulin dimers along the microtubule, and was asymmetric, reflecting the structural polarity of the microtubule. We argue that these frictional forces arise from breaking bonds between the motor domains and the microtubule, and they limit the speed and efficiency of kinesin.

**1872-Pos****Structural Basis for Microtubule Binding by the Anti-Parallel Microtubule Crosslinker PRC1 (Protein Regulator of Cytokinesis-1)**Radhika Subramanian<sup>1</sup>, Elizabeth M. Wilson-Kubalek<sup>2</sup>, Elizabeth A. Campbell<sup>1</sup>, Matthew J. Bick<sup>1</sup>, Seth A. Drast<sup>1</sup>, Ronald A. Milligan<sup>2</sup>, Tarun M. Kapoor<sup>1</sup>.<sup>1</sup>Rockefeller University, New York, NY, USA, <sup>2</sup>The Scripps Research Institute, La Jolla, CA, USA.

Successful cytokinesis is essential for maintaining genome stability. The central spindle, an array of antiparallel microtubules formed prior to cytokinesis, is required for complete furrow ingression to form two daughter cells. Members of the conserved MAP65 family (Ase1, AtMAP-65, PRC1) play a key role in organizing microtubules in the central spindle. Invitro studies indicate that members of this protein family can crosslink microtubules with an anti-parallel orientation bias. We have combined structural methods and single molecule fluorescence to understand the structural basis for biased crosslinking by the human MAP65 protein PRC1.

We describe the crystal structure of the conserved domain of PRC1. This domain is capable of binding microtubules (Kd ~ 6  $\mu$ M), but shows no sequence similarity to other known microtubule binding proteins. This domain has a spectrin fold, a motif ubiquitous in proteins interacting with the actin cytoskeleton, but not seen previously in microtubule binding proteins. Cryo-EM and helical image analysis yielded a 3D map of PRC1 bound to microtubules. Docking the crystal structure into the EM map gives us a structural view of PRC1-microtubule interactions.

PRC1 also has a proteolytically sensitive Lys/Arg rich domain that is thought to regulate microtubule binding. Single-molecule assays reveal that the presence of this domain allows the protein to undergo long diffusional sliding on microtubules. Constructs in which this domain is truncated show reduced interaction-lifetime and crosslinking efficiency.

These results suggests that the two domains act together to achieve biased anti-parallel crosslinking. We propose a model where oriented binding and polarity bias is achieved by the spectrin domain and the positively charged flexible domain enhances the probability of biased crosslinking by increasing the dwell time of PRC1 on microtubules via a diffusive binding mechanism.

**1873-Pos****Trapping and Polymerization of Tubulin Within Phospholipid Vesicles**

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Microtubules are cylindrical aggregates (diameter ~25 nm) of the protein tubulin. They play important roles in cellular structure, protein trafficking, cell motility, intracellular transport, meiosis, and mitosis. They can switch stochastically between periods of tubulin polymerization and dissolution, termed dynamic instability, and can individually apply an extensive force at a significant distance on the cellular scale (Howard and Hyman 2003). Previous light microscopy has been used to characterize the forces that produce membrane distortions when microtubules assemble inside lipid vesicles (Fygenson et al. 1997; Emsellem et al. 1998).

We have examined this phenomenon by cryo-EM to gain insights on membrane deformation and to visualize the detailed structure of the microtubule ends as they are constrained near the inner membrane surface. Vesicles need to be of sufficient size, typically 1  $\mu$ m diameter, to contain sufficient tubulin to form a microtubule long enough to span the vesicle. This has presented a challenge given limitations on the thickness of EM samples.

To date, we have visualized microtubule polymers in even small vesicles that often produce significant distortion, along with microtubules that form outside the vesicles. Quite unexpectedly, it appears that in certain cases the vesicles are deformed by the microtubule, sometimes with long protrusions, while in other cases in vesicles of similar size, the microtubules are deformed by the vesicles, with a number of sharp bends. This presents an opportunity to gain insights on the factors involved in both lipid bilayer and microtubule strength and flexibility. Ultimately we will image these samples using electron tomography to describe all of the effects in three dimensions. Determination of the nature of the dynamic instability and microtubule growth and vesicle tensile strength resulting from microtubule elongation will provide insight into the nature of cellular architecture, plasticity, and deformation.